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POLYMERIC MATERIALS FOR PROTECTION AGAINST CHEMICAL AND BIOLOGICAL CONTAMINANTS

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The objective of this project was to attach N-halamine functional groups to various polymeric materials to be used as oxidizing agents to render the materials biocidal and resistant to chemical agents. The biocidal objective was successfully achieved for the textile materials nylon and polyester, as well as for a water-borne acrylic polyurethane paint and for functionalized polystrene beads to be used for water disinfection in cartridge filters. Activities of the materials against chemical agents remain to be tested. Common features of all of the materials developed are the abilities to stabilize oxidative chlorine for long periods of time, to be regenerated by exposure to aqueous solutions of free chlorine (bleach), and to inactivate pathogenic microorganisms upon surface contact. It is envisioned that the various materials developed in this research project will be useful to the military in a variety of applications such as in defense against biological and chemical agents in medical facilities, barracks, tents, transport vehicles, and water treatment devices.

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ABSTRACT

The objective of this project was to attach N-halamine functional groups to various polymeric materials to be used as oxidizing agents to render the materials biocidal and resistant to chemical agents. The biocidal objective was successfully achieved for the textile materials Nylon and polyester, as well as for a water-borne acrylic polyurethane paint and for functionalized polystyrene beads to be used for water disinfection in cartridge filters. Activities of the materials against chemical agents remain to be tested. Common features of all of the materials developed are the abilities to stabilize oxidative chlorine for long periods of time, to be regenerated by exposure to aqueous solutions of free chlorine (bleach), and to inactivate pathogenic microorganisms upon surface contact. It is envisioned that the various materials developed in this research project will be useful to the military in a variety of applications such as in defense against biological and chemical agents in medical facilities, barracks, tents, transport vehicles, and water treatment devices.

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SUMMARY

The objectives of the project, namely to attach N-halamine functional groups to various polymeric materials to be used as oxidizing agents to render the materials biocidal and possibly resistant to chemical agents were clearly met. The polymeric materials modified and tested included Nylon, polyester, a polyurethane paint, and polystyrene beads.

Biocidal cyclic N-chloramine moieties were covalently bonded to Nylon 66. These moieties, which included hydantoins, oxazolidinones, and imidazolidinones, were stable during at least three months of dry storage, and their antimicrobial activities, once lost by reaction with reducing sodium thiosulfate, could be regenerated by exposure to free chlorine. Biocidal swatch tests showed that the Nylon fabrics containing N-chlorinated hydantoin functional groups provided a 7.2 log reduction of *Staphylococcus aureus* and a 7.1 log reduction of *Escherichia coli* at a contact time of only 10 minutes. Antimicrobial Nylon should find a variety of important uses such as in clothing, carpets, sutures, brushes, etc.

Polyester fabrics were modified by covalently linking heterocyclic hydantoin moieties, which could be halogenated, to the surfaces of the polyester fibers. Antimicrobial activity against *S. aureus* and *E. coli* was introduced into the fabrics and fibers by exposure to a source of oxidative chlorine (chlorine bleach) which converted the heterocyclic precursor moieties into N-chloramine functionalities. The antimicrobial activity could be repeatedly regenerated following its loss on challenge with suspensions of bacteria by further washing with aqueous oxidative chlorine. The treatment conditions

did not significantly affect the strength of the fibers. Biocidal polyester fabrics, fibers, and other materials potentially will be effective in reducing, or eliminating entirely, pathogenic microorganisms and odor-causing microorganisms which directly contact them.

A novel precursor N-halamine diol monomer was prepared which was copolymerized with a commercial waterborne acrylic polyol and a commercial isocyanate to produce a polyurethane coating which could be applied to a broad variety of surfaces. The coating was then chlorinated with a source of free chlorine, such as bleach, to render it biocidal. Once the coating lost its chlorine loading, and hence its biocidal activity, regeneration was possible by further exposure to free chlorine. In one experimental observation a coating on a wall at Tyndall AFB retained its biocidal activity for more than six months. The biocidal coating should have many applications, including in medical facilities, in food preparation areas, in prevention of biofouling in aqueous and humid environments, etc.

The biocidal polymers poly-1,3-dichloro-5-methyl-5-(4'-vinylphenyl)hydantoin, poly-1,3-dibromo-5-methyl-5-(4'-vinylphenyl)hydantoin, and the monochlorinated derivative were prepared as insoluble porous beads. Halogen stability, rechargeability, and efficacy against pathogens (*Staphylococcus aureus, Escherichia coli* O157:H7, MS2 virus, and poliovirus) in a water filtration application were evaluated. While the polymers previously prepared in powdered granular solid form were effective against a wide variety of pathogens in contact times of a few seconds, the flow rates of water through cartridge filters containing them were often diminished due to clogging problems. Furthermore, the fine particles could be partially

aerosolized in a manufacturing facility thus causing a potential hazard for workers in the facility. The porous beads, prepared entirely by heterogeneous reactions, overcame these limitations while maintaining outstanding biocidal efficacies. The beads contained in small cartridge filters should be very useful to the military for treatment of field water in remote areas.

The research performed in this project was sucessful for four primary classes of polymeric materials. This work, coupled with that done prior to this project, and that now ongoing in these laboratories, demonstrates the great importance of functionalizing polymers with N-halamine moieties in order to instill biocidal activity.

INTRODUCTION

Work in these laboratories since 1980 has focused on the syntheses and testing of a series of heterocyclic compounds which can be referred to as N-halamines. These compounds contain nitrogen-chlorine or nitrogen-bromine moieties which can be stabilized by alkyl substitution on the carbon atom(s) adjacent to the nitrogen atom(s) contained in the heterocyclic ring. The compounds developed in these laboratories are biocidal, the mechanism of action being direct contact of the molecules with pathogenic cells allowing donation of oxidative chlorine or bromine atoms to receptors in the cell. Cell inactivation then is most likely produced by oxidation in a manner similar to that produced by free chlorine or free bromine. The reader is referred to two articles co-authored by the principal investigator which review much of the prior work on N-halamine biocidal compounds.[1,2]

More recent work in these laboratories has involved functionalizing useful polymeric materials with N-halamine moieties in order to render them biocidal. This was the focus of the present project, the materials being Nylon, polyester, polyurethane paint, and polystyrene porous beads. The results for each of these materials will be discussed independently in the following sections. The discussion will be organized in the form of independent publications; the substance of all four are in some stage of the publication process at this time.[3-6]

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BIOCIDAL NYLON

ABSTRACT

Biocidal cyclic N-chloramine moieties were covalently bonded to Nylon 66. These moieties, which included hydantoins, oxazolidinones, and imidazolidinones, were stable during at least three months of dry storage, and their antimicrobial activities, once lost by reaction with reducing sodium thiosulfate, could be regenerated by exposure to free chlorine. Biocidal swatch tests showed that the Nylon fabrics containing N-chlorinated hydantoin functional groups provided a 7.2 log reduction of *Staphylococcus aureus* and a 7.1 log reduction of *Escherichia coli* at a contact time of only 10 minutes. Antimicrobial Nylon should find a variety of important uses such as in clothing, carpets, sutures, brushes, etc.

INTRODUCTION

Some microorganisms are highly undesirable as a cause of odors, skin irritation, and illness. The odor on clothing arises primarily as a result of bacteria and fungi which are growing in the perspiration and on the skin cells which are in the clothing. Bacteria and fungi are deposited on carpets through the normal traffic of people and animals, food and beverages spilled on the carpet, and animal and infant excreta. Frequent, long-lasting local infections may be brought about by Nylon surgical sutures incorporated into tissues and soaked with liquids being potential culture media for bacteria.[1] Therefore, it would be useful if products made of Nylon could have antimicrobial activity.

Recently, in response to the demand for a safe antimicrobial and deodorizing treatment, chemical methods have been proposed using as an antimicrobial component, halamines which are not toxic, such as N-halohydantoins.[2-4] These types of moieties have successfully been incorporated into fabrics produced from cellulose.[5-7] In the present work, three types of cyclic chloramine moieties were chemically bound to the surfaces of Nylon 66 fabrics and fibers using the chemistry shown in Figure 1.

EXPERIMENTAL

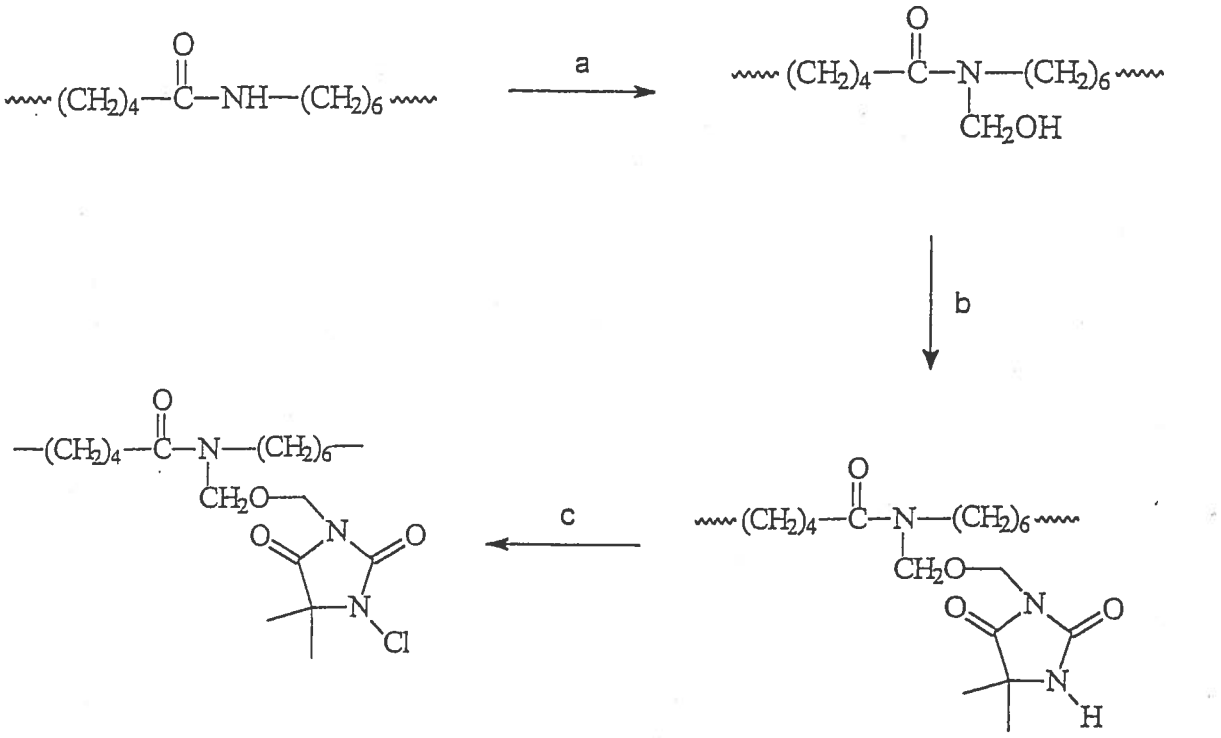
Treatment Process

An example of the treatment process used in this study follows. A

2.0 g sample of Nylon 66 fabric (or fibers) was soaked in 200 ml of 10% concentration of formaldehyde solution under basic conditions (0.5 N NaOH) at 80 C for 2 h. The fabric or fibers were washed with distilled water until a neutral pH was obtained. Then the fabric or fibers now containing a hydroxymethyl functional group at the amide nitrogen of Nylon 66 were placed in a treatment bath containing 10 g of 4-hydroxymethyl-4-ethyl-2-oxazolidinone prepared as in reference 4, 0.5 g of $MgCl_2$, 0.2 g Triton X-100 wetting agent, and 200 ml of distilled water held at pH 3.5 and 80 C for 30 min. The treated Nylon 66 now containing the oxazolidinone functional group on the surface of the material was then cured in an oven at 130 C for 15 min. After curing, it was washed with detergent at 50 C for 30 min. Then the treated material was soaked in dilute bleach (0.75 % active chlorine) at ambient temperature for 3 h before antimicrobial testing. Nylon 66 materials were also treated with 3-hydroxymethyl-2,2,5,5-tetramethylimidazolidin-4-one and 3- and 1-hydroxymethyl-5,5-dimethylhydantoin using analogous procedures (see Scheme 1).

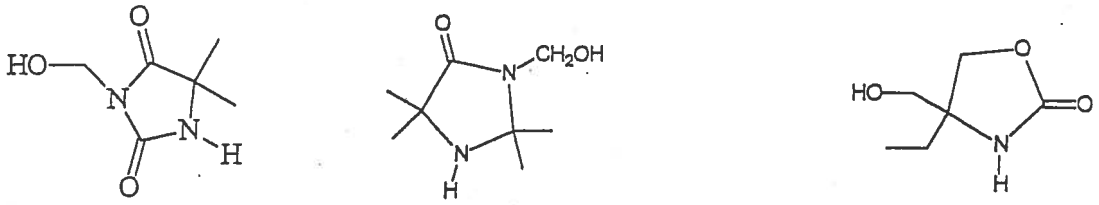
Titration Analyses of Chlorine on the Fabric

The dry fabrics were stored in closed plastic bags at room temperature for a period up to three months. Over a selected time interval, a piece of the fabric was removed from the stock bag and cut into strips. The strips were dipped in 100 mL of distilled water in a beaker, and about 1 mg of potassium iodide was then added. The mixture was held for 5 h to ensure complete reaction of potassium iodide with the combined chlorine moiety. Then the solution containing the fabric was analyzed by the standard iodometric



a = formaldehyde

b =



hydroxymethylhydantoin (as shown in above scheme)

hydroxymethylimidazolidinone

hydroxymethyloxazolidinone

c = free chlorine

Figure 1. Reaction Scheme Used in Producing the Antimicrobial Nylon Samples

titration method. The equation used to calculate the concentration of chlorine (in mg) on the surface of one side of fabric is:

$$W_{Cl} = (V \times N \times 35.45) / (S \times 4)$$

where, W_{Cl} = mg of chlorine on one side of 1.00 cm^2 of fabric, V = volume of titrant (mL), N = normality of sodium thiosulfate titrant, and S = sample surface area (in cm^2).

Each analysis was run in triplicate, and the three titration results were always within 5% precision.

Antibacterial Tests

The antibacterial efficacies of the biocidal Nylon 66 fibers and fabric swatches were evaluated as follows. The test and control (unchlorinated) fiber samples were tested quantitatively for antibacterial activity using a column bacteria test. In this test, the sample was placed in a sterile glass buret or pipet, i.e. the column. The empty bed volume of the sample was measured in order to calculate the contact time of the inoculum with the sample. This was done by measuring the volume of water which exactly filled the region of the column containing the fibers. The sample was tested to ensure that no free, unbound chlorine was present. This was achieved by repeatedly washing the sample in the column with chlorine demand free water, and testing the resultant wash water with chlorine indicator strips, until the strips indicated a concentration of less than 0.2 mg/L free chlorine in the effluent. A known volume of inoculum containing about 9.2×10^7

CFU/mL of *Staphylococcus aureus* in pH 7 buffer, typically 1.0 mL, was passed through the column and collected during which time the flow rate was recorded. A 25.0 μL aliquot of the collected solution was quenched with an equal volume of 0.02 N sodium thiosulfate, and then a 25.0 μL sample of this mixture was plated onto a nutrient agar plate. The remaining bacterial solution was then passed through the column once again, and again it was sampled and plated onto agar. This procedure was repeated typically for a total of six passes of the 1.0 mL inoculum. The resultant aliquots were then incubated for a period of 48 h. The bacteria colonies were counted at 24 h and 48 h providing information with regard to the contact time required to produce an efficient antibacterial activity. An identical column containing unchlorinated fibers was used as a control.

For the fabric swatches antibacterial tests were conducted using AATCC Method 100. In the method, sized and shaped treated swatches were placed in sterile petri dishes. A known volume of inoculum containing bacteria (about 10^7 or 10^8 CFU/ml(*staphylococcus aureus* 1.1 x 10^7 - 5.0 x 10^8 and *Escherichia coli* 2.0 x 10^7)) in pH 7 buffer solution was used. Complete absorption of the bacterial solution was required with no free solution being available. Swatches of identical fabric, but containing no biocidal finish, acted as controls. After inoculation, each swatch was transferred into a sterile wide mouthed glass vessel containing 0.02 N sodium thiosulfate to quench disinfectant action. The vessel and contents were shaken, and an aliquot of the resulting mixture was removed. A set of serial dilutions were performed using pH 7 buffer. A 25.0 μL aliquot of each dilution was then plated on nutrient agar and incubated for a period of 24-48 h. Bacterial counting was performed after 24 h and 48 h of incubation.

RESULTS AND DISCUSSION

Stability of Chlorine on the Fabric

The results of the stability tests for Nylon treated with the hydroxymethylhydantoin monomer and chlorinated are tabulated in Table 1.

Table 1. Titration Results for Chlorine on the Surface of Nylon Fabric Treated with a Hyroxymethylhydantoin Monomer

Storage Time (days)	Cl on Surface ($mg/cm^{2)}$	% Chlorine
1	0.0119	100
3	0.0107	89.6
7	0.0104	87.0
14	0.0101	84.4
28	0.0096	80.5
61	0.0091	76.6
94	0.0081	67.5

It was observed that the retention of chlorine on the surface of the fabric was about 70% after three months of storage. The analogous experiments with the other two monomers were not performed. However, it has been observed here that N-chlorooxazolidinones and N-chloroimidazolidinones are inherently more stable to loss of chlorine than are N-chlorohydantoins, so it

can be anticipated that Nylon treated with the other two monomers will retain its chlorine for periods exceeding three months.

Antibacterial Efficacies

The Nylon 66 fibers which were treated with the hydroxymethylhydantoin and subsequently chlorinated provided an 8 log reduction (complete inactivation) of *Staphylococcus aureus* within 16.8 sec; whereas, the unchlorinated control fibers gave no reduction of the bacteria even at a contact time of 71 sec. Thus, an authentic inactivation of the bacteria occurred rather than just filtration. The results of the swatch testing are shown in Tables 2 and 3.

Table 2. Bactericidal Performance of Nylon 66 Swatches Treated with Chlorinated Hydroxymethylhydantoin

Bacterium	Contact Time (min)	Log Reduction[a]
S. aureus	10	7.2
E. coli	10	7.1
S. aureus	30	7.2
E. coli	30	7.1

[a]The control swatches showed only a reduction of about 1 log.

Table 3. Bactericidal Performance of Nylon 66 Swatches Treated with Three Different N-Halamines against *S. aureus*

Treatment Monomer[a]	Chlorination	Challenge (Logs)	Contact Time (min)	Reduction (logs)
HMHY	Yes	8.7	60	8.7
HMHY	No	8.7	60	1.3
HMOX	Yes	8.6	60	6.1
HMOX	No	8.6	60	0.6
HMIM	Yes	8.6	60	5.5
HMIM	No	8.6	60	0.8

[a]HMHY = hydroxymethylhydantoin

HMOX = hydroxymethyloxazolidinone

HMIM = hydroxymethylimidazolidinone

It is clear from the results in the two tables that the treated Nylon 66 fibers and swatches were bactericidal with the hydantoin treatment being the most effective, at least in terms of efficacy at the contact times studied for the freshly prepared samples. However, it should be noted, that for N-halamine moieties, the biocidal efficacy as assessed by contact time necessary to achieve a given log inactivation is inversely related to the stability of the N-Cl or N-Br covalent bond. In other words, compounds or materials containing cyclic N-halamine moieties which require longer contact times for antimicrobial activity will be more stable to loss of halogen and possess enhanced long-term biocidal activity.

An experiment was also performed to determine whether the antibacterial activity could be regenerated after its loss. Fabric samples were treated with the hydroxymethylhydantoin compound and chlorinated. Test and unchlorinated control fabric samples were tested quantitatively for antibacterial activity against *Staphylococcus aureus* (1.23×10^8 CFU) using the swatch bacteria test. The two samples were then exposed to 100 mL of 0.02 N sodium thiosulfate for one min. Then a second chlorination was performed on the previously chlorinated sample, and the swatch test was performed a second time for both the chlorinated and unchlorinated samples. The results are shown in Table 4.

Table 4. Regeneration of Antibacterial Activity against *S. aureus*[a]

Chlorination	Challenge Time (min)	Log Reduction
First	60	8.1
Second	60	8.1

[a]After the first bacterial challenge the swatch was dechlorinated using sodium thiosulfate as a reducing agent, and then rechlorinated, followed by a second bacterial challenge.

It is clear that antibacterial activity was restored to the previously chlorinated sample by a second chlorination.

The nitrogen-chlorine bond is very stable in these treated Nylon 66 materials. The mechanism of action must be a direct contact of the bacterial cell with the bound chlorine atom resulting in transfer of the chlorine and subsequent cell inactivation. The bound chlorine atom is subject to loss mechanisms upon chemical interaction with reducing agents, but as noted above, it can be replenished by subsequent exposure to a source of free chlorine such as bleach.

Work in these laboratories is currently being directed toward the biocidal treatment of commercial Nylon articles using techniques similar to those discussed above as well as other important fiber, textile, and industrial materials.

CONCLUSIONS

This study has demonstrated that Nylon 66 fibers can be rendered antimicrobial by chemically bonding a heterocyclic N-halamine functional group to the Nylon 66 molecule at the amide nitrogen using formaldehyde as a linking agent. The treated materials have been shown to be bactericidal against the bacteria *Staphylococcus aureus* and *Escherichia coli*. Upon loss of the antimicrobial chlorine atom, activity can be restored by exposure to a source of free chlorine such as bleach. It is anticipated that this technology will be useful in rendering such commercial products antimicrobial as clothing, carpets, surgical sutures, and brush bristles.

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BIOCIDAL POLYESTER

ABSTRACT

Polyester fabrics were modified by covalently linking heterocyclic moieties, which could be halogenated, to the surfaces of the polyester fibers. Antimicrobial activity was introduced into the fabrics and fibers by exposure to a source of oxidative chlorine (chlorine bleach) which converted the heterocyclic precursor moieties into N-chloramine functionalities. The antimicrobial activity could be repeatedly regenerated following its loss on challenge with suspensions of bacteria by further washing with aqueous oxidative chlorine. Biocidal polyester fabrics, fibers, and other materials potentially will be effective in reducing, or eliminating entirely, pathogenic microorganisms and odor-causing microorganisms which directly contact them.

INTRODUCTION

A very desirable property to be introduced into textile fabrics is antimicrobial activity for the obvious uses in medical applications, as well as for reducing noxious odor in clothing, carpets, hygienic pads, and air filters. A considerable amount of research effort has been expended in recent years in attempts to render the polymers employed in manufacturing textile products biocidal. Most efforts have involved the use of coating, grafting, impregnation, and blending technologies which can yield antibacterial, or at least bacteriostatic, properties. However, it is common with these technologies that the biocidal function is short-lived and non-regenerable upon exposure to multiple wash cycles or to reactive chemicals. The optimum biocidal textile should be one in which the biocidal functionality is imparted to the host polymer through an irreversible chemical reaction to produce covalent bonds without causing significant deterioration of the desirable and necessary properties of the host polymer. If the biocidal property is lost over a period of time due to inherent instability, then it is important that it can be restored through some type of regeneration process.

A biocidal functionality for polymers which satisfies the requirements mentioned above is the cyclic N-halamine moiety. Extensive work in these laboratories over the past decade[1] has established that N-halamine groups such as N-halo hydantoins, oxazolidinones, and imidazolidinones can be covalently attached to a variety of polymers used in water disinfection applications,[2-4] surface coatings,[5-7] and elastomers.[8] Sun and coworkers, in pioneering work on textile fabrics, have extended the technology to its use in

rendering fabrics containing cellulose (cotton and cotton blends) biocidal.[9,10] More recently, it has been demonstrated that nylon can also be rendered biocidal with similar technology.[11,12] As will be discussed herein, it is now evident that poly(ethylene terephthalate), PET, can also be functionalized with a biocidal N-halamine moiety utilizing similar chemistry to that employed for cellulose and nylon.

Several recent reports concerning the introduction of an antibacterial functionality into PET have appeared. Buchenska has shown that some antibacterial activity can be imparted to PET fibers which have been grafted with acrylic acid and subsequently treated with antibiotics; the antibiotics are slowly released into solutions.[13] Kang and coworkers have produced chitosan-grafted and quaternized-chitosan-grafted PET following a glow-discharge graft of acrylic acid onto the PET; the treated PET was able to inhibit the growth of bacteria after long contact times (6 h).[14] Yang and coworkers have treated 100% polyester fabric in a range finishing and batch exhaustion process with 2-hydroxy-2',4,4'-trichloro-diphenyl ether and showed the treated fabric to have weak bactericidal efficacy against *Staphylococcus aureus* and *Escherichia coli* before and after multiple washes.[15] Sun and coworkers have recently demonstrated that the novel monomers 3-allyl-5,5-dimethylhydantoin and 1-acryloyl-2,2,5,5-tetramethylimidazolidin-4-one can be grafted onto PET in a continuous finishing process.[16,17] Upon subsequent chlorination, the resulting fabrics containing the N-chloramine functionality become biocidal and can withstand multiple washing cycles while retaining activity. More important, once activity is lost, the treated PET can be rechlorinated and regain its biocidal efficacy.[16,17] The present study will demonstrate an alternate means of

creating the N-halamine biocidal functionality on PET. A portion of the PET chains on the surface of the fibers will be interupted through alkaline hydrolysis in aqueous ammonia, followed by reaction of the resulting amide fragments with 3-hydroxymethyl-5,5-dimethylhydantoin, and subsequent chlorination with sodium hypochlorite (Figure 2). It will be demonstrated that the resulting treated PET is bactericidal and capable of being regenerated once the initial chlorine charge is exhausted.

EXPERIMENTAL

Treatment Process

Square swatches (3x3 in) of PET fabric (100% Dacron Type 54 purchased from Test Fabrics, Inc., Middlesex, NJ) were washed according to American Association of Textile Chemists and Colorists(AATCC) Test Method 124 before use. The cleaned swatches were soaked in saturated ammonium hydroxide solution (29.6%) at 34°C for a variable time of 0.5 to 3.0 h followed by three distilled water rinses. A treating bath was prepared which contained 5.0 g of MDMH (a mixture of 3-hydroxymethyl-5,5-dimethylhydantoin and 1-hydroxymethyl-5,5-dimethylhydantoin (Dantoin® purchased from the Lonza Chemical Company, Fairlawn, NJ)), 0.6 g of magnesium chloride as a catalyst, 0.2 g of Triton X-100 as a wetting agent, and 100 mL of distilled water. The pH of the bath was adjusted to 2.5 with 1% sulfuric acid solution. The fabric swatches were soaked in the bath at 80°C for 30 min. After drying in air, they were cured at 140°C for 2.0 hours under a nitrogen atmosphere. Then they were washed with chlorine-demand-free (CDF) water. The swatches were rendered biocidal by soaking in

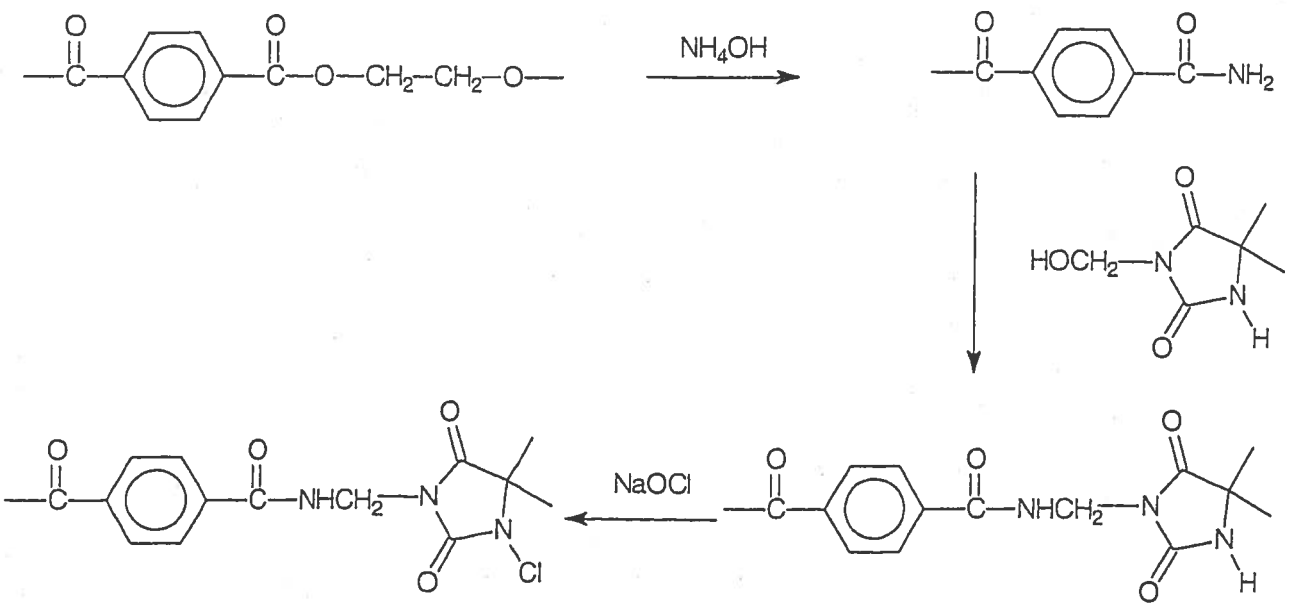

Figure 2. Reaction Sequence Used in Producing the Biocidal PET Fabric

a solution of free chlorine (50% Clorox®) which contained 2.6% of sodium hypochlorite for a variable time of 5 min to 8.0 h at room temperature. Then they were rinsed with CDF water until free chlorine could not be detected in the effluent (<0.2 mg/L). Following drying in air, they were tested for antimicrobial efficacy. The chemical reactions used in the treatment process are shown in Figure 2.

Biocidal Efficacy

Swatches of test and control fabrics were tested quantitatively for antibacterial activity using a modified version of AATCC Method 100. In the method, sized and shaped treated swatches were placed in sterile petri dishes. A known volume of inoculum containing bacteria (Gram-positive *Staphylococcus aureus* (ATCC 5368) or Gram-negative *Escherichia coli* (ATCC 2666)) at a concentration of about 10^8 CFU/mL in pH 7 phosphate buffer solution was used. For the inoculation procedure, complete absoption of the bacterial solution was required with no free solution being available. Swatches of unchlorinated, but otherwise identical fabric, were employed as controls. Each swatch was inoculated with a known volume of inoculum ensuring even distribution and, after a measured contact time, was transferred into a sterile wide mouthed glass vessel. Following the transfer, 0.02 N sodium thiosulfate was added to quench further biocidal action. The vessel and contents were shaken, and an aliquot of the resulting mixture was removed. Following a set of serial dilutions with pH 7 phosphate buffer, a 0.025 mL aliquot of each dilution was plated on Nutrient agar and incubated for a period of 48 hours. Bacterial counting was performed after 24 hours and 48 hours of incubation.

Analyses of Chlorine on Fabric

Two types of experiments were performed. In one, the biocidal PET fabric was examined for loss of chlorine during storage. In the other, the fabric was exposed to sodium thiosulfate (0.00375 N) so as to reduce all of the oxidative chlorine, and then it was rechlorinated several times as described in the Treatment Section. A standard iodometric/thiosulfate titration procedure was employed to measure the chlorine content of the fabric. Strips of known surface area were soaked in 100 mL of distilled water containg 0.1 g of potassium iodide, 1 mL of phthalate buffer (pH 4), and 3 drops of 1% starch in a flask. The flask was sealed after purging with nitrogen gas, and the mixture was stirred at room temperature for 8 h. The resulting blue solution containing the strips of fabric was then titrated with standard sodium thiosulfate to a clear endpoint. The equation used to calculate the mg of Cl on the surface of the fabric is:

$$W_{Cl} = (V \times N \times 35.45)/(S \times 2)$$

Where W_{Cl} is the mg/cm^2 of Cl bonded to the surface of the fabric, V and N are the volume and normality, respectively, of the sodium thiosulfate, and S is the surface area in cm^2 of the swatch.

Tensile Strength Test

An Instron Model 1122 Tensile Tester was employed to determine the maximum load and percent elongation of the treated PET fibers upon

reaching the point of breakage. A total of 10 one-inch fibers were tested for each sample with the results averaged. The tests were conducted at 21°C and 65% relative humidity. The full-scale load on the constant-rate-of-extension Instron was 50.0 lbf, and the crosshead speed employed was 10 in/min. The samples were treated with ammonium hydroxide for 0.5 hours, 1.0 hour, 1.5 hours, 2.0 hours, and 3.0 hours, corresponding to samples PETH-1, PETH-2, PETH-3, PETH-4, and PETH-5 in Table 6. "PET" refers to poly(ethylene terephthalate); "H" refers to subsquent treatment with MDMH. The samples tested were not chlorinated.

RESULTS AND DISCUSSION

The key reaction step in the conversion of PET fabric into a form which can be rendered bactericidal is the formation of amide fragments on the surfaces of the fibers. This was accomplished by hydrolysis of a portion of the PET ester linkages and subsequent reaction with ammonia. The hydrolysis step can be effected with other bases such as dilute sodium hydroxide, followed by exposure to ammonium hydroxide to form the amide fragments. However, ammonium hydroxide works well in its dual role of inducing ester hydrolysis and formation of the amide fragments. Also, being a weak base, less fragmentation occurs with the ammonia, which leads to less deterioration of the strength of the fibers than is the case with the stronger base. Treatment of polyester fabric with alkaline solutions is already commonly employed during commercial processing. It was found that time of exposure in the bath containing the ammonium hydroxide (0.5 - 3.0 h) did not affect the biocidal efficacy of the fabric, nor its tensile strength, as will be discussed later. Also, it was found that variation of chlorination

conditions for the treated fabric (concentration of bleach in the range 50-100%; time of chlorination in the range 5 min to 8.0 h) did not affect the biocidal efficacy.

The results in Table 5 show that when a very high challenge load of *S. aureus*

Table 5. Antibacterial Swatch Testing after Different Contact Times

Sample[a]	Contact Time (min)[b]	Antibacterial Performance (log reduction)
PETHCl	30	6.9
PETH	30	1.9
PETHCl	20	6.6
PETH	20	1.9
PETHCl	10	5.3
PETH	10	0.9
PETHCl	5	4.3
PETH	5	2.0

[a] "Cl" indicates a chlorinated sample having antibacterial activity; absence of "Cl" indicates an unchlorinated control.
[b] The challenge of *S. aureus* was 1.3 x 10^9 CFU (9.1 logs) per 6.45 cm^2 swatch.

(9.1 logs) was employed, the chlorinated samples showed reasonable antibacterial activity (6.9 log inactivation) at a contact time of 30 min; there was some efficacy (4.3 log inactivation) even at the shortest contact time (5 min) tested. If the contact time is extended to 60 min, an 8.5 log reduction of *S. aureus* has been observed. In a similar, but limited, study of the efficacy of the treated PET against *E. coli*, a 5.7 log reduction (100%) was observed at a contact time of 10 min, but the reduction dropped to 64% at a contact time of 5 min.

Table 6. Antibacterial Swatch Testing after Variable Times of Dry Storage

Sample	Time after Preparation (days)	Challenge of *S. aureus* (log)[a]	Microbiological Performance (log reduction)
PETHCl	1	8.4	8.4
PETH	1	8.4	0.0
PETHCl	3	8.3	8.3
PETH	3	8.3	1.2
PETHCl	7	9.0	6.8
PETH	7	9.0	0.3
PETHCl	14	9.0	6.8
PETH	14	9.0	0.2

[a] The challenge contact time was 60 min.

The efficacy results in this study compare favorably with those obtained for PET treated with other N-chloramine functionalities,[16,17] and are much superior to those reported elsewhere for which other treatment techniques were employed.[13-15]

Tables 6 and 7 present data relevant to the stability of the chlorine bonded to the hydantoin moiety during dry storage. From Table 6 it is observed that the efficacy of the treated textile fabric against *S. aureus* declined only slightly (from 8.4 log inactivation to 6.8 log inactivation) over a 2 week period of storage in a sealed plastic bag at room temperature in the absence of light.

Table 7. Stability of Chlorine Bonded on Halamine Moiety after Dry Storage

Time of Storage (days)	Chlorine (mg/cm^2) on Fabric
1	6.47×10^{-3}
3	5.70×10^{-3}
7	4.76×10^{-3}
14	2.98×10^{-3}

The data in Table 7 indicate that the treated PET fabric lost about 54% of its chlorine over the 2 weeks, and that after storage for 1 day, it contained 1.10×10^{17} Cl atoms/cm^2. This chlorine loading was adequate to completely

inactivate the 2.5 x 10^8 CFU/swatch (having surface area 6.45 cm^2) of *S. aureus* during the 60 min contact time.

Tables 8 and 9 address the regenerative property of the biocidal PET fabric.

Table 8. Regeneration of Antibacterial Activity

Sample	Chlorination	Microbiological Performance (log reduction)[a]
PETHCl-1	First: 50% Clorox®	9.0
PETH	None	0.3
PETHCl-2	Second: 50% Clorox®	9.0
PETH	None	0.2

[a] The challenge of *S. aureus* was 1.0 x 10^9 CFU (9.0 logs) per 6.45 cm^2 swatch for a contact time of 60 min.

Table 8 shows that the biocidal efficacy of the treated PET could be completely recovered after being destroyed by the reducing agent sodium thiosulfate by rechlorination under the same conditions as originally employed. Table 9 indicates that insignificant changes in chlorine atoms/cm^2 occur over 5 cycles of reduction with sodium thiosulfate followed by reoxidation with chlorine bleach.

Table 9. Chlorine on Regenerated PET Fabric

Repetitive Chlorination	Chlorine (mg/cm^2) on Fabric
1st	6.47×10^{-3}
2nd	6.00×10^{-3}
3rd	5.79×10^{-3}
4th	5.62×10^{-3}
5th	5.70×10^{-3}

The data in Table 10 were generated to test whether the ammonia treatment of the PET caused deterioration of the fibers.

Table 10. Tensile Properties of PET Fibers by Single-strand Method

Sample[a]	Displacement at Maximum (lbf)	Strain at Maximum (%)	Tensile Strength (lbf)
PET original[b]	0.57	57	1.10
PETH-1	0.43	43	1.06
PETH-2	0.55	55	1.09
PETH-3	0.46	46	1.06
PETH-4	0.52	52	1.07
PETH-5	0.44	44	1.01

[a] The samples were treated with ammonium hydroxide for different times (see Experimental Section).

[b] The sample was not treated, ie. a control.

The standard deviations in the data were such that it can be stated that there was no significant difference between the treated fibers and the PETH control fibers, even when long-term exposure to the ammonium hydroxide solution was employed. In other words, the treatment conditions did not significantly affect the strength of the fibers. The chlorinated samples were not tested, but any significant weakening of the PETH fibers should have occurred under the ester hydrolyses conditions, ie. under the conditions of exposure to ammonium hydroxide.

Generally the biocidal efficacies of N-halamine compounds are inversely related to the strengths of their nitrogen-halogen covalent bonds.[18] This appears to be true even for N-halamine compounds which liberate very little free halogen into aqueous solution. These compounds with very strong N-Cl bonds are thought to inactivate pathogens by a direct contact mechanism in which the oxidative chlorine atom is transferred directly to the cell, rather than following dissociation of the N-Cl bond through hydrolysis to form "free chlorine" which then becomes the active biocide. Thus it is conceivable that an N-halamine moiety other than the hydantoin discussed above could provide a more rapid inactivation of the pathogens, but have less shelf life, or vice versa. In fact, this notion is borne out by recent results from these laboratories. Using the N-halamine precursors 4-hydroxymethyl-4-ethyl-2-oxazolidinone and 3-hydroxymethyl-2,2,5,5-tetramethylimidazolidin-4-one, and the same treatment procedure as reported in the Experimental Section for the hydantoin derivative, we have been able to produce the corresponding biocidal PET fabrics.[19] These biocidal PET fabrics require somewhat longer contact times for complete inactivation of *S. aureus* than does the hydantoin

derivative, but they retain chlorine for a longer period. This is as expected since the N-Cl bond is stronger for the oxazolidinone and imidazolidinone moieties than for the hydantoin.

CONCLUSIONS

This study has demonstrated that PET fabrics can be rendered antimicrobial by chemically bonding heterocyclic N-halamine functional groups to the polyester molecule following fragmentation of a portion of its ester linkages utilizing ammonia for hydrolysis and conversion to amide fragments without significantly reducing the strengths of the fibers. The treated materials have been shown to be bactericidal against the bacteria *Staphylococcus aureus* and *Escherichia coli.* Upon loss of the antimicrobial chlorine atom, activity can be restored by exposure to a source of free chlorine such as bleach. It is anticipated that this technology will be useful in rendering such commercial products as clothing antimicrobial, and for reducing, or eliminating, noxious odors in polyester products.

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BIOCIDAL POLYURETHANE PAINT

Abstract

A novel precursor N-halamine diol monomer was prepared which was copolymerized with a commercial waterborne acrylic polyol and a commercial isocyanate to produce a polyurethane coating which could be applied to a broad variety of surfaces. The coating was then chlorinated with a source of free chlorine, such as bleach, to render it biocidal. Once the coating lost its chlorine loading, and hence its biocidal activity, regeneration was possible by further exposure to free chlorine. In one experimental observation a coating on a wall at Tyndall AFB retained its biocidal activity for more than six months. The biocidal coating should have many applications, including in medical facilities, in food preparation areas, in prevention of biofouling in aqueous and humid environments, etc.

Introduction

Work in these laboratories since 1980 has focused on the development of novel biocidal N-halamine derivatives.[1] Water-soluble cyclic N-halamine derivatives such as 1,3-dihalo-5,5-dimethylhydantoin and halogenated isocyanurates (eg. Trichlor and Dichlor) have been employed as biocides for industrial and recreational water uses for many years, but the water-soluble N-halamine compounds produced in these laboratories (oxazolidinones and imidazolidinones) are superior because of their long-term stabilities in aqueous solution and in dry storage (see structures in Figure 3). This exceptional stability is a result of their chemical structures; all have electron-donating alkyl groups substituted on the heterocyclic rings adjacent to the oxidative N-Cl or N-Br moieties which hinder the release of "free halogen" into aqueous solution. The combined N-halamines thus serve as the contact biocides.

Although combined N-halamine monomers generally require longer contact times at a given halogen concentration than does "free halogen" to inactivate pathogens, it has been demonstrated in these laboratories that it is possible to concentrate N-halamine moieties on insoluble polymers, thus producing a substantial reservoir of combined halogen for enhanced disinfection purposes. Furthermore, the functionalized N-halamine polymers are superior in overall performance (taking into account biocidal efficacy, stability at varying pH's and in the presence of organic receptors, rechargeability, lack of toxicity, and cost) to other biocidal polymers which have been developed over the years, some of which are in the commercial sector, such as halogenated poly-styrene-divinylbenzenesulfonamides[2],

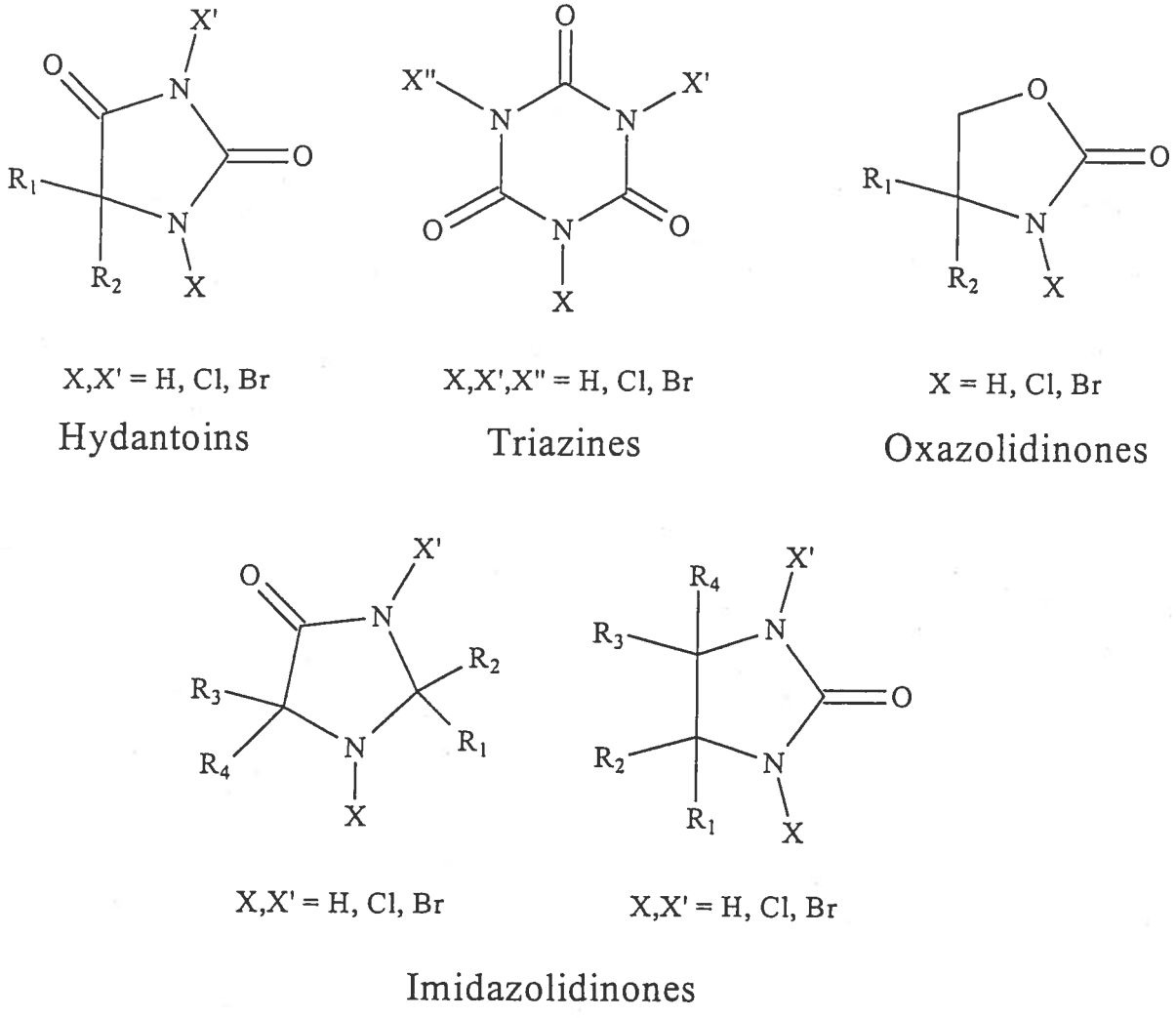


Figure 3. Heterocyclic Moieties for Use in Producing Biocidal Polymers

polymeric phosphonium materials[3], and polymeric quaternary ammonium compounds[4].

Several commercial polymers have been functionalized with N-halamine moieties rendering them biocidal upon surface contact with pathogens. These include cellulose[5,6], nylon[6,7], PET[6,8], Kraton rubber[9], and various surface coatings[10]. But to date, the most important N-halamine polymers developed, because of their potential for economical disinfection of potable water, thus improving world health, are the N-halogenated poly-styrenehydantoins.[11-15] These products were granular solids which were insoluble in water, but they could be chlorinated or brominated by adding free chlorine or free bromine, respectively. The final products were amorphous solids which were insoluble in water and could be packed into glass columns which functioned as a cartridge filters. It was observed that the filters inactivated numerous species of bacteria, fungi, and even rotavirus in only seconds of contact time in flowing water.[11-15] Also, it was observed that the columns did not leach out decomposition products into the water,[14] and that the free chlorine and bromine concentrations leached into the flowing water were less than 0.1 mg/L and less than 2.0 mg/L, respectively. Furthermore, once the halogen supply was exhausted through various loss processes, it could be replenished on the polymers by simply exposing them to flowing aqueous free halogen (eg. sodium hypochlorite bleach for the chlorinated derivative). It appeared that the chlorinated polymer would be useful for potable water disinfection applications throughout the world, and that the brominated polymer would work well in disinfecting recreational water sources. Recently the products have been produced in the form of porous beads to enhance flow properties.

This work represents an extension of technology developed in these laboratories to the preparation of biocidal polyurethane coatings through functionalization of a reactive diol with a hydantoin moiety which can then be copolymerized with commercial polyols and isocyanates to form the polyurethane. An application of free halogen (eg. with household bleach) will then render the coating biocidal. The concept is illustrated in Figure 4, and the actual diol which has been developed in this work is shown in Figure 5.

Experimental

Preparation of Diol Monomer

The unhalogenated diol monomer was prepared by reaction of 5,5-dimethylhydantoin, diethanolamine, and formaldehyde at ambient temperature in methanol as a solvent. Alternatively, it could be prepared by reaction of 3-hydroxymethyl-5,5-dimethylhydantoin with diethanolamine in methanol at 75°C. The water byproduct and methanol solvent were removed for characterization purposes by vacuum evaporation. The viscous residue produced was then dissolved in ethyl acetate, and anhydrous sodium sulfate was added for further drying purposes. Following removal of the sodium sulfate by filtration, the solution was refrigerated. After 12 hours, a white solid product precipitated from the ethyl acetate solution. The product, which was removed by filtration from the cold solution, exhibited a melting point of 74-76°C and was produced in 61-84 % yield; it was identified as 5,5-dimethyl-3-(N,N-di-ß-hydroxyethylaminomethyl)hydantoin. ^{1}H NMR (DMSO-d_6) ∂ 1.28 (6H), 2.65 (4H), 3.40 (4H), 4.31 (2H), 4.39 (2H), 8.28 (1H); ^{13}C NMR

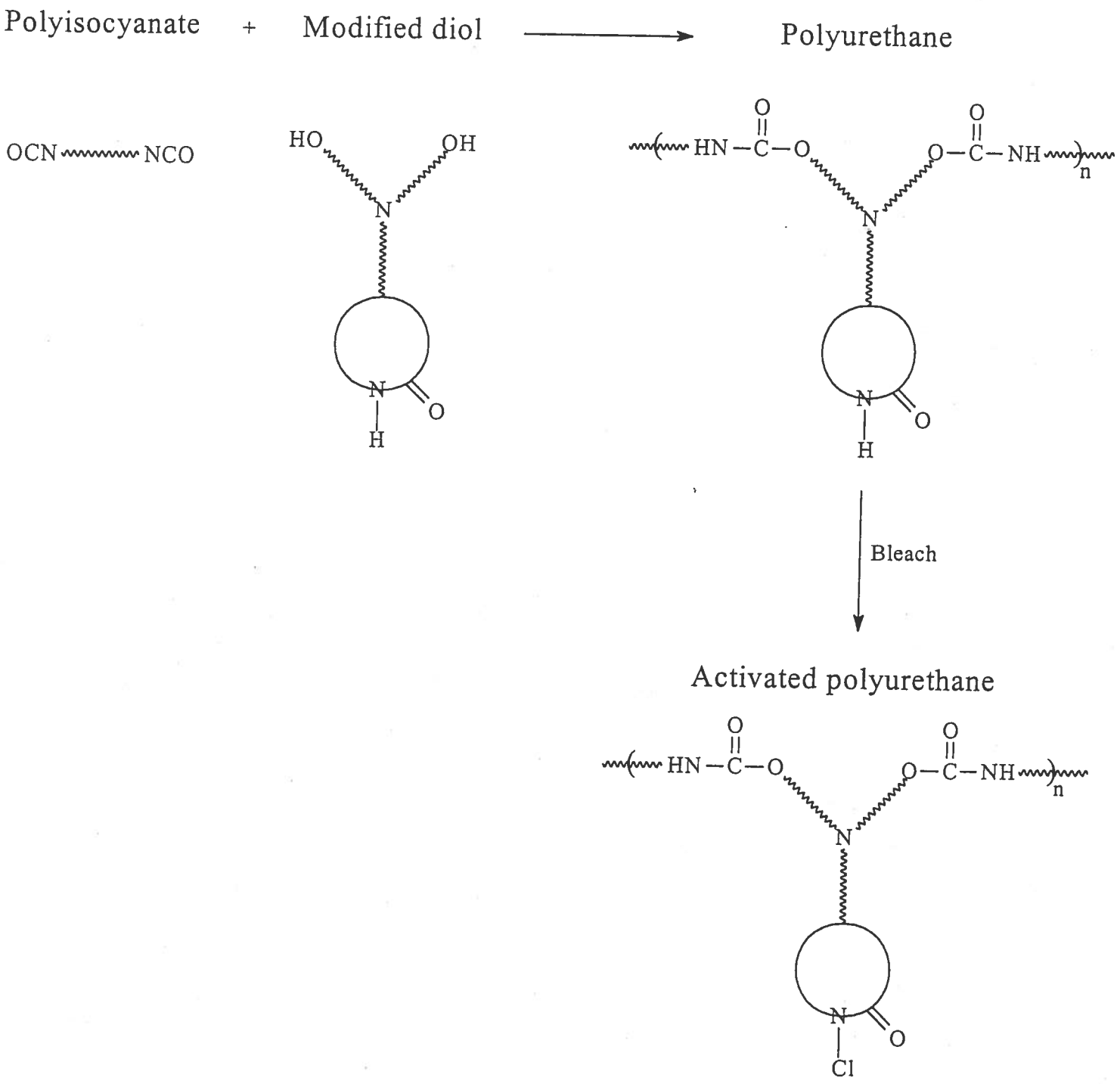
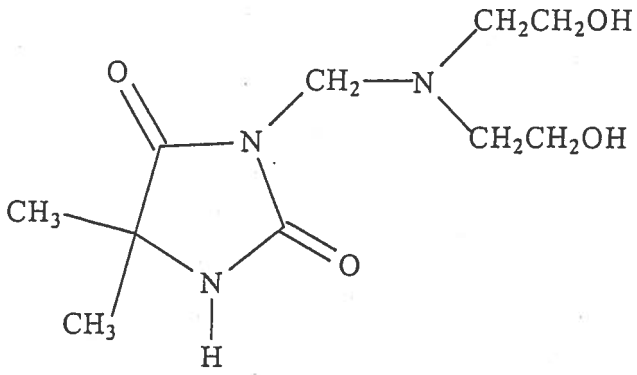


Figure 4. Schematic of a Biocidal Polyurethane Coating

Diol Monomer Developed for Polyurethane Coatings



0.7 g diol monomer + 10 g waterborne acrylic polyol + 2.45 g isocyanate + 2.1 g water

↓

Polyurethane

↓ Bleach

Biocidal Polyurethane

Figure 5. Diol Monomer Developed for Polyurethane Coatings

(DMSO d_6) ∂ 24.8, 54.5, 57.6, 57.8, 59.2, 156.2, 178.7; IR (Kbr) 1295, 1346, 1439, 1710, 1764, 2814, 2974, 3227, 3474
cm^{-1}.

Preparation and testing of Polyurethane Coatings

To 10.0 g of commercial waterborne acrylic polyol formulation was added 0.7 g of the unhalogenated diol monomer, prepared as described above, with stirring until dissolution was complete. Then 2.45 g of commercial isocyanate formulation was thoroughly mixed in, followed by the addition and mixing of 2.10 g distilled, deionized water. The resulting formulation was immediately spread onto the surfaces of several plastic Petri dishes which were dried in air at ambient temperature. The coatings were dry to the touch within 4-5 h, but were allowed to cure further overnight at ambient temperature before further treatment. The coatings were then chlorinated by exposure to commercial bleach (5.25 % sodium hypochlorite) at several concentrations for 3-12 h. After rinsing thoroughly with chlorine-demand-free water, the coatings were dried in air for 6 h and then analyzed for bound oxidative chlorine using an iodometric thiosulfate titration procedure. Other coatings prepared in the same manner at the same time (cut to squares of 6.45 cm^2 area) were challenged with *Staphylococcus aureus* for contact times of 2 h. This was done by placing 25 μL of bacterial solution between two coated squares. Following quenching of disinfectant action with 0.02 N sodium thiosulfate in a vortexed solution in a beaker, serial dilutions of the vortexed solution were plated onto trypticase soy agar, incubated for 48 h at 37°C, and colony counts were made. Unchlorinated coatings served

as controls. The analytical and microbiological evaluations were performed as a function of chlorination concentration and of time following chlorination.

In another experiment, strips of unhalogenated coatings were deposited on the wall of a restroom at Tyndall AFB; half of the strips were chlorinated with bleach; the other half were not chlorinated to serve as controls. The strips were challenged with bacteria after rinsing and drying, and then again 6 months later without rechlorination. Finally, polycarbonate strips were coated with the polyurethane and placed in a biofilm reactor at the Center for Biofilm Engineering at Montana State University; uncoated strips served as controls. Water containing nutrients which support biofilm growth was flowed through the reactor at a shear stress simulating a flow of 1 ft/s in a 4 in pipe. After 5 weeks substantial biofilm development had occurred on all strips. At that time the water was doped with 1.0-1.2 mg/L of free chlorine, and the flow was continued for 5 more weeks with the behavior of the biofilms on the strips continuously monitored microbiologically.

Results and Discussion

The data are shown in tables 11 and 12. Table 11 shows that a complete inactivation of the bacteria (>4.5 logs) in 2 h contact time was obtained after 10 and 5 % bleach solutions were used for chlorination for 3 h, and a 3.0 log inactivation occurred following exposure of the coating to 1 % bleach solution for 3 h. This is consistent with the trend of Cl atoms/cm^2 for the identical samples determined analytically.

Table 11. Function of Chlorination Concentration

Clorox Concentration %	Cl Atoms/cm^2 Surface	Log Reduction *S. aureus*
10	1.34 x 10^{17}	>4.5 (no growth)
5	9.13 x 10^{16}	>4.5 (no growth)
1	3.69 x 10^{16}	3.0

Table 12 shows that the coatings retained their biocidal efficacies for at least 14 d (longer times were not tested in this particular experiment). It has also been demonstrated that biocidal efficacy can be regenerated once lost by reexposure to free chlorine solutions.

Table 12. Coating Chlorine Loadings and Biocidal Efficacies as a Function of Time Following Chlorination with 100 % Clorox for 12 Hours

Time after Chlorination in Days	Cl Atoms/cm^2 Surface	Log Reduction *S. aureus*
0.25	3.53 x 10^{17}	>4.7 (no growth)
4.0	6.78 x 10^{16}	>4.7 (no growth)
14.0	2.33 x 10^{16}	>4.7 (no growth)

The results of the wall experiment performed at Tyndall AFB were very gratifying. No viable bacteria were cultured from the polyurethane strips which had been chlorinated originally even after 6 months without recharging. Also gratifying were the results of the biofilm reactor study at Montana

State University. When the small amounts of free chlorine (1.0-1.2 mg/L) were present in the flowing water, the strips containing the polyurethane coating yielded 1-2 logs less biofilm microorganisms than did the polycarbonate strips not containing a polyurethane coating. This is dramatically illustrated in the photograph shown in Figure 6; strip A is a control strip with no polyurethane coating, while strips B and C contain the chlorinated coating.

CONCLUSIONS

A novel hydantoinyl diol monomer has been prepared in a simple, inexpensive process. The monomer has been copolymerized with a commercial waterborne acrylic polyol and a commercial isocyanate to produce a polyurethane coating. The coating can be chlorinated with a source of free chlorine, such as bleach, to render it biocidal. The coating loses its chlorine loading gradually, but it can be regenerated by further exposure to free chlorine. The biocidal coating should have many applications, including in medical facilities, in food preparation areas, in prevention of biofouling, etc.

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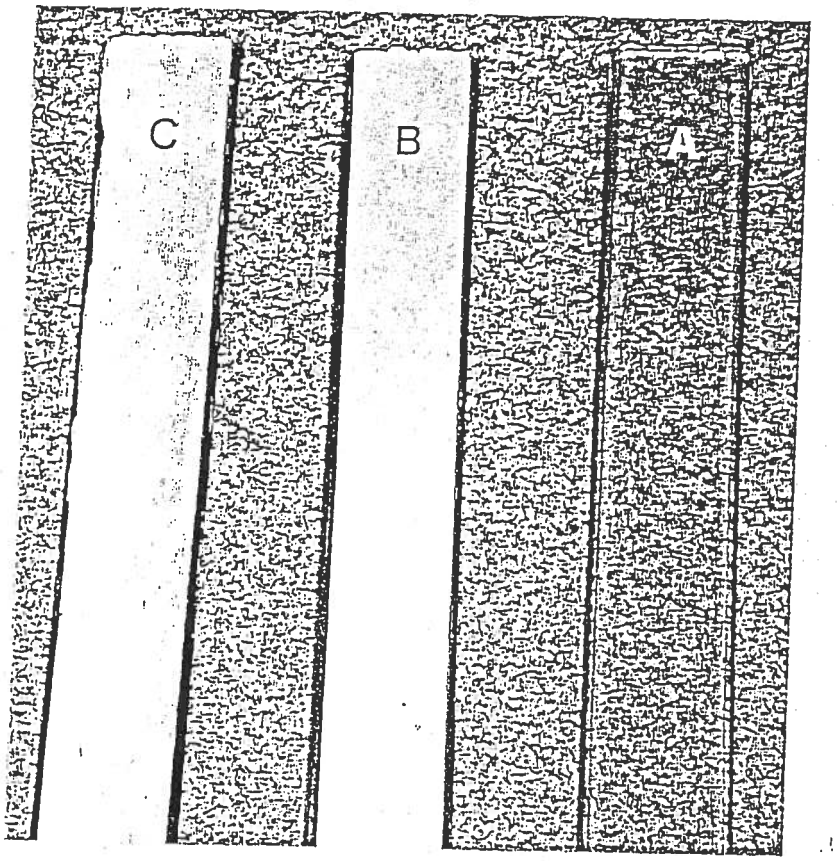

Figure 6. Polycarbonate Slides Subjected to Biofilm Formation

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BIOCIDAL POLYSTYRENE BEADS FOR WATER TREATMENT

Abstract

The biocidal polymers poly-1,3-dichloro-5-methyl-5-(4'-vinylphenyl)hydantoin, poly-1,3-dibromo-5-methyl-5-(4'-vinylphenyl)hydantoin, and the monochlorinated derivative were prepared as insoluble porous beads. Halogen stability, rechargeability, and efficacy against pathogens (*Staphylococcus aureus, Escherichia coli* O157:H7, MS2 virus, and poliovirus) in a water filtration application were evaluated. While the polymers previously prepared in powdered granular solid form were effective against a wide variety of pathogens in contact times of a few seconds, the flow rates of water through cartridge filters containing them were often diminished due to clogging problems. Furthermore, the fine particles could be partially aerosolized in a manufacturing facility thus causing a potential hazard for workers in the facility. The porous beads, prepared entirely by heterogeneous reactions, overcame these limitations while maintaining outstanding biocidal efficacies.

Introduction

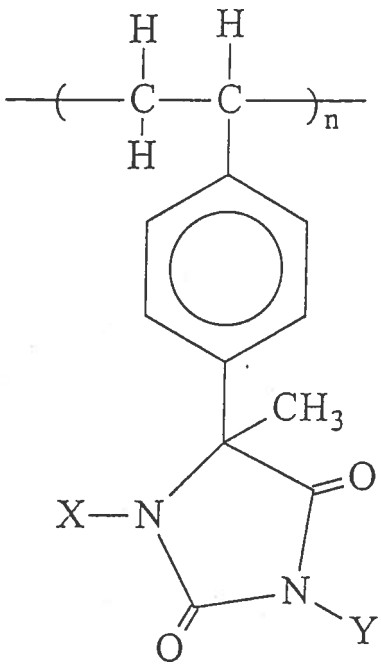
Work in these laboratories since 1980 has focused on the development of novel biocidal N-halamine derivatives.[1] Water-soluble cyclic N-halamine derivatives such as 1,3-dihalo-5,5-dimethylhydantoin and halogenated isocyanurates (eg. Trichlor and Dichlor) have been employed as biocides for industrial and recreational water uses for many years, but the water-soluble N-halamine compounds produced in these laboratories (oxazolidinones and imidazolidinones) are superior because of their long-term stabilities in aqueous solution and in dry storage. This exceptional stability is a result of their chemical structures; all have electron-donating alkyl groups substituted on the heterocyclic rings adjacent to the oxidative N-Cl or N-Br moieties which hinder the release of "free halogen" into aqueous solution. The combined N-halamines thus serve as the contact biocides.

Although combined N-halamine monomers generally require longer contact times at a given halogen concentration than does "free halogen" to inactivate pathogens, it has been demonstrated in these laboratories that it is possible to concentrate N-halamine moieties on insoluble polymers, thus producing a substantial reservoir of combined halogen for enhanced disinfection purposes. Furthermore, the functionalized N-halamine polymers may be superior in overall performance (taking into account biocidal efficacy, stability at varying pH's and in the presence of organic receptors, rechargeability, lack of toxicity, and cost) to other biocidal polymers which have been developed over the years, some of which are in the commercial sector, such as halogenated poly-styrene-divinylbenzenesulfonamides[2],

polymeric phosphonium materials[3], and polymeric quaternary ammonium compounds[4].

Several commercial polymers have been functionalized with N-halamine moieties rendering them biocidal upon surface contact with pathogens. These include cellulose[5,6], nylon[6,7], PET[6,8], Kraton rubber[9], and various surface coatings[10]. But to date, the most important N-halamine polymers developed, because of their potential for economical disinfection of potable water, thus improving world health, are the N-halogenated poly-styrenehydantoins (Figure 7).[11-15] In previous work it was shown that the biocidal polymers poly-1,3-dichloro-5-methyl-5-(4'-vinylphenyl)hydantoin (Poly1-Cl) and poly-1,3-dibromo-5-methyl-5-(4'-vinylphenyl)hydantoin (Poly1-Br) could be prepared by a three-step procedure.[11,12] In the first step commercial poly-styrene having low cross-linking was dissolved in an organic solvent such as carbon disulfide, and a Friedel-Crafts acylation was performed utilizing acetyl chloride and the catalyst aluminum chloride. The poly-4-vinylacetophenone thus produced was then dissolved in an ethanol/water mixture, and it was reacted in the second step with potassium cyanide and ammonium carbonate in a pressure reactor to produce poly-5-methyl-5-(4'-vinylphenyl)hydantoin. This product was a granular solid which was insoluble in water, but it could be chlorinated or brominated at 10°C by slowly adding free chlorine or free bromine, respectively, under alkaline conditions. The final products were amorphous solids which were insoluble in water and could be packed into glass columns which functioned as a cartridge filters.

It was observed that the filters inactivated numerous species of bacteria, fungi, and even rotavirus in only seconds of contact time in flowing



X= Cl, Br; Y= Cl, Br, H

Figure 7. Structural Formula of the Halogenated Polystyrene Hydantoin

water.[11-15] Also, it was observed that the columns did not leach out decomposition products into the water,[14] and that the free chlorine and bromine concentrations leached into the flowing water were less than 0.1 mg/L and less than 2.0 mg/L, respectively. Furthermore, once the halogen supply was exhausted through various loss processes, it could be replenished on the polymers by simply exposing them to flowing aqueous free halogen (eg. sodium hypochlorite bleach for Poly1-Cl). It appeared that the chlorinated polymer would be useful for potable water disinfection applications throughout the world, and that the brominated polymer would work well in disinfecting recreational water sources.

However, there were limitations inherent in the polymers produced as described above. First, the particle size could not be controlled in the three step synthesis; the particles varied in diameter from less than 10 μm to several hundred μm. A large portion of the particles were small; these migrated to the end of the filter causing plugging and some deposition into the disinfected effluent water. Also, larger particles tended to break into smaller particles over an extended use period. Second, the irregular particle distribution led to flow reproducibility problems from batch to batch. Third, the dried solids exhibited noticeable halogen odor when stored in enclosed containers such as do many commercial halogenated resin materials. A buildup of free halogen in the storage container could lead to occupational exposure for workers in an industrial setting.

The limitations have now been circumvented by converting highly cross-linked poly-styrene porous beads of uniform size into biocidal polymers while maintaining particle size control, and by carefully controlling the halogenation

conditions by appropriate pH adjustments. The methods and some performance data will be described herein.

Experimental Methods

Preparation of Poly1-Cl Porous Beads

Typically 10 g of porous beads of 5.6% cross-linked polystyrene obtained from Suqing Group (Jiangyin, Jiangsu, PRC) having particle sizes in the range 250 to 600 μm and pore sizes of about 50 nm were cleaned by soaking them in reagent-grade acetone for 2 h at 25°C and then by passing two 30 mL portions of acetone through them in a filter funnel. Following drying to constant weight in air at 25°C, the polystyrene beads were converted by heterogeneous reactions first to poly-4-vinylacetophenone porous beads, then to poly-5-methyl-5-(4'-vinylphenylhydantoin) (PSH) porous beads, and finally to highly cross-linked, porous poly-1,3-dichloro-5-methyl-5-(4'-vinylphenyl)hydantoin (Poly1-Cl) beads utilizing the chemistry described earlier for the powdered material.[11,12,16] It was found that the chlorination reaction step could be performed either with chlorine gas bubbled into a flask containing the PSH beads in alkaline solution, or by the addition of sodium hypochlorite solution (bleach). The final chlorine loading on the Poly1-Cl could be controlled by pH adjustments. A pH of 8.0 - 8.5 was generally used to achieve a weight percent Cl loading of 14 - 18, at which loading the "chlorine odor" emitted from the beads was minimal. For example, in one such batch iodometric/thiosulfate titration indicated that the chlorine loading was 16.9% by weight. The infrared spectrum of the beads from this batch (crushed to a powder) in a KBr pellet exhibited prominent bands at 1751 and 1805 cm^{-1},

in good agreement with those of the powdered poly-1,3-dichloro-5-methyl-5-(4'-vinylphenyl)hydantoin disclosed in U.S. Patent 5,490,983.[16] Furthermore, the beads retained their shapes throughout the three reaction steps and increased somewhat in size (to 400 - 800 μm) due to swelling.

Preparation of Poly1-Br Porous Beads

Poly-5-methyl-5-(4'-vinylphenyl)hydantoin (PSH) porous beads (5.0 g) were suspended in a flask containing 50 mL of 2 N NaOH. To the stirred suspension, liquid bromine was added dropwise at 25°C over a period of 10 min. Then the pH was adjusted to 6.4 by the addition of 4 N acetic acid, and the mixture was stirred at 25°C without the further addition of bromine for 1 h. The brominated beads were then filtered and washed 5 times with 100 mL portions of tap water and dried in air at 25°C for 8 h. An iodometric/thiosulfate titration indicated that the beads contained a 36.8 percent by weight loading of bromine An infrared spectrum of a small sample of the beads (crushed to a powder) in a KBr pellet exhibited prominent bands at 1724 and 1779 cm^{-1}, in good agreement with those of powdered poly-1,3-dibromo-5-methyl-5-(4'-vinylphenyl)hydantoin prepared earlier starting from soluble poly-styrene pellets, indicative of an efficient heterogeneous reaction of bromine with the insoluble, highly cross-linked, porous PSH beads.

Bacteria Challenges

The porous Poly1-Cl and Poly1-Br beads prepared as described above with chlorine loading of about 20% and bromine loading of about 37% and

contained in glass columns were tested for biocidal activity against two bacterial pathogens contained in water. For example, in one test about 3.9 g of chlorinated beads were packed into a glass column having inside diameter of 1.3 cm to a length of about 7.6 cm; the empty bed volume was measured to be 3.3 mL. Two types of control samples were prepared. In one control, unchlorinated PSH beads were employed. In the other, Poly1-Cl beads were reduced with 1.5 N sodium thiosulfate solution so as to maintain the size and pore sizes of the beads as produced upon chlorination.

After washing the Poly1-Cl column with chlorine-demand-free water until less than 0.2 mg/L of free chlorine could be detected in the effluent (about 1 L of chlorine-demand-free water or of pH 7.0 buffer was generally required), an aqueous solution of 50 mL of pH 7.0 phosphate-buffered, demand-free water containing about 6.9×10^6 CFU (colony forming units)/mL of the Gram positive bacterium *Staphylococcus aureus* (ATCC 6538) was pumped through the column at a measured flow rate of about 3.0 mL/s. The effluent was quenched with 0.02 N sodium thiosulfate before plating of serial dilutions on nutrient agar. Colony counts were performed after incubation at 37^0C for 24 and 48 h. A second bacterial pathogen studied was Gram negative *Escherichia coli* O157:H7 (ATCC 43895) at a concentration of about 8.5×10^6 CFU/mL. The detailed procedures for microbiological analyses in these laboratories have been documented.[17]

A column of the same dimensions packed with the highly brominated beads (empty bed volume of 3.1 mL) was prepared. After washing the column with about 1 L of demand-free water until less than 1 mg/L of free bromine could be detected in the effluent, an aqueous solution of 50 mL of pH 7.0

phosphate-buffered, demand-free water containing $6.9x10^6$ CFU/mL of the Gram positive bacterium *S. aureus* (ATCC 6538) was pumped through the column at a measured flow rate of about 3.0 mL/s. The effluent was quenched with 0.02 N sodium thiosulfate before plating. The Gram negative bacterium *Escherichia coli* O157:H7 (ATCC 43895) at a concentration of $8.5x10^6$ CFU/mL was also employed as a challenge pathogen to the Poly1-Br beads. The control column containing unhalogenated PSH beads was subjected to the same challenge conditions.

Following each experiment, the biocidal bead columns were rinsed with chlorine-demand-free water and then sterilized and refurbished by exposure to flowing dilute solutions of free chlorine or free bromine. The control bead columns were sterilized in an autoclave following each experiment.

Virus Challenges

Columns of Poly1-Cl beads were evaluated for efficacy against MS2 and polio viruses in flowing water. In one experiment about two portions of 19 g each of chlorinated beads (each containing 14 % chlorine loading) were packed into 25 mL sterile pipets, each column having empty bed volumes of about 6.0 mL. Stock solutions of MS2 virus (ATCC15597-B1) were prepared using the agar overlay method suggested for use with ATCC 15597-B1; *E. coli* ATCC 15597 was employed as the host for the MS2 virus. The viral titer was determined for the stock solution. Then dilutions were made into distilled water containing 0.3 g/L of sea salts (NaCl, KCl, $CaCl_2$, and $MgCl_2$) and buffered to pH values of 6.5, 7.0, and 7.5. The columns were rinsed with distilled water until less than 0.1 mg/L of free chlorine could be detected in

the effluent. Then the viral suspension was pumped through the columns at measured flow rates varying from 0.065 to 1.85 mL/s, resulting in contact times of 91 to 3.2 s, respectively. A portion of the effluent at each flow rate was sampled, immediately quenched with 0.02 N sodium thiosulfate to destroy any residual free chlorine leached out of the column, and subjected to analysis for viable MS2 virus using the agar overlay method mentioned above. Plates were quantitated after 24 h incubation at 37°C which allowed the determination of log reductions of pfu (plaque forming units)/mL as a function of contact time with the Poly1-Cl beads. A second portion of effluent at each flow rate was analyzed for the presence of free chlorine using a Hach DR/4000 spectrophotometer and the DPD method.

Similar experiments were performed using poliovirus as the challenge pathogen. In these experiments the columns contained from 18 to 29 g of porous Poly1-Cl beads. Lower flow rates (0.04 - 0.17 mL/s), ie. longer contact times (46 - 428 s), were employed in these experiments since poliovirus proved to be more resistant to inactivation than did MS2 virus, and comparisons were made of effluent immediately quenched with plating media as opposed to delayed quenching (plating) so as to expose the effluent virus particles to low concentrations of leached free chlorine. Sodium thiosulfate could not be used in the experiments with poliovirus for quecheing inactivation because it damages the host cells in the assay procedure. The poliovirus (ATCC VR-193) was propagated in Vero cells (African Green Monkey kidney cells - ATCC CCL 81) in the presence of complete Dulbeccos Modified Eagles Medium (cDMEM) and 5% fetal bovine serium (FBS) under 5-10% CO_2 at 37°C for 2-3 d. The poliovirus assay procedure was an agar overlay method involving Vero cells in cDMEM and Eagles Minimum Essential Medium (MEM)

with pfu being counted to determine the number of infective viral particles present per mL of effluent from the columns.

Stability and Rechargeability Studies

The Poly1-Cl beads were tested for retention of chlorine under dry storage. Beads initially containing 17.16 % chlorine loading were stored in a vacuum desiccator. Periodically over a 90 d storage time, samples were subjected to oxidative chlorine analysis using an iodometric/thiosulfate titration procedure.

Poly1-Cl beads were also tested for their durability after simulated usage/recharge cycles. In this experiment beads (10 g) initially containing a chlorine loading of 17.4% were subjected to 100 cycles of partial neutralization with sodium thiosulfate (100 mL of 2.67% $Na_2S_2O_3$ for 5 min while stirring, then rinsing, and vacuum filtration) and recharging (100 mL of 21% Clorox Ultra® for 15 min while stirring, then rinsing, and vacuum filtration). At cycles 55, 75, and 100, 100% Clorox Ultra® was employed to ensure maximum chlorination, and the pH was adjusted to 8.5 using 20% acetic acid. Small samples of dried beads were periodically analyzed for their chlorine loadings (after cycles 1, 55, 75, and 100) using an iodometric/thiosulfate titration procedure.

Results and Discussion

The 6.9×10^6 CFU/mL of the Gram positive bacterium *S. aureus* (ATCC 6538) were inactivated in one pass through the column of Poly1-Cl beads (with a

20% chlorine loading), i.e. a 6.8 log reduction in a contact time of less than or equal to 1.1 s. The same result was achieved with the Gram negative bacterium *E. coli* O157:H7 (ATCC 43895) at a concentration of 8.5×10^6 CFU/mL, i.e. a 6.9 log reduction in a contact time of less than or equal to 1.1 s. The control columns containing unhalogenated PSH beads or reduced Poly1-Cl beads gave no reduction of either bacterium in a contact time of 1.6 s when the same concentrations of the inoculums were employed. Thus it can be concluded that the bacteria were inactivated, not merely removed by filtration. In one experiment with the Poly1-Cl beads in which the sample weight was reduced to 1.5 g (empty bed volume of 1.5 mL) and the chlorine loading was only 12.5%, it was found that a complete inactivation of *S. aureus* (6.7 logs) could be obtained in a contact time of only 0.5 s. Furthermore, for 3.06 g of the beads which were primarily the monochlorinated derivative (10.5% by weight chlorine) with empty bed volume of 3.84 mL, a challenge with 50 mL of *S. aureus* at a concentration of about 1.1×10^7 CFU/mL at a flow rate of 3.0 mL/second, provided a complete 7.1 log reduction in a contact time of less than or equal to 1.3 seconds. The bacterial suspensions in these experiments were in chlorine-demand-free water. Somewhat longer contact times would be expected for suspensions containing heavy chlorine demand; although these experiments were not performed for the beads, the original Poly1-Cl powder was shown to be very effective in disinfecting USEPA worst-case water containing sea salts and heavy organic load.[12] The Poly1-Br beads gave similar results for the two bacterial pathogens. All of the *S. aureus* bacteria were inactivated in one pass through the column, i.e. a 6.8 log reduction in a contact time of less than or equal to 1.0 second. The same result was achieved with the Gram negative bacterium *E. coli* O157:H7, i.e. a 6.9 log reduction in a contact time

of less than or equal to 1.1 second. Thus, both types of porous beads were extremely effective against the two bacterial pathogens in very brief contact times even when the halogen loadings were less than the theoretical maximum (24.9% for Cl; 42.8% Br). In fact, the results were comparable to those described earlier for Poly1-Cl and Poly1-Br powder samples containing much higher surface areas (see Table 13);[11-16] greater flow rates were

Table 13. Bactericidal Efficacies of Poly1-Cl and Poly1-Br in Bead Versus Powder Form

Column Material	*S. aureus*[a] Contact time (s)[b]	*S. aureus*[a] Log reduction[b]	*E. coli*[a] Contact time (s)[b]	*E. coli*[a] Log reduction[b]
Poly1-Cl Beads[c]	1.1	>6.8	1.1	>6.9
Poly1-Cl Powder[d]	5.0	>6.7	4.9	>8.5
Poly1-Br Beads[c]	1.0	>6.8	1.1	>6.9
Poly1-Br Powder[d]	2.4	>6.2	2.0	>6.1

[a] In pH 7.0 chlorine-demand-free water at 22°C.

[b] Lowest contact time evaluated; all bacteria were inactivated.

[c] A Cl loading of 20.0%; a Br loading of 36.8% - this work.

[d] A Cl loading of 22.5%; a Br loading of ca. 40% - reference 12.

employed in this work for the beads. In other experiments more than 150 L of *E. coli* suspension at 6 logs/mL were passed through 20 g of the Poly1-Cl beads in 10 L increments without any breakthrough of live bacteria.[18]

In general, virus particles are more difficult to inactivate than are bacteria by free halogen and N-halamines. However, the Poly1-Cl beads functioned extremely well against the MS2 virus particles. In numerous experiments at pH's 6.5-7.5 in which the contact times ranged down from 91 s to 3.2 s, the lowest contact time tested, no MS2 particles survived a single pass through the column, at a typical log reduction of about 5.0. Any small amount of free chlorine leaching out of the column (typically less than 0.1 mg/L) was quenched immediately with sodium thiosulfate. The results were less spectacular for poliovirus which is known to be quite resistant to chlorine inactivation. Typically, contact times of the order of 2 min were necessary to achieve complete inactivation (4-5 logs) upon one pass through the column when the effluent was immediately plated on assay media. A contact time of 107 s provided only a 1.4 log inactivation of the virus particles in this manner. However, it was unexpectedly discovered that when the effluent was not immediately plated on media, much better results were obtained. For example, after a contact time in the column of only 46 s, 1.8×10^5 pfu/mL were completely inactivated after residing in the collection reservoir for 60 min. Yet the free chlorine concentration in the reservoir was measured to be less than 0.05 mg/L. Separate experiments on similar poliovirus concentrations in water containing 0.05 mg/L of free chlorine have established that little or no inactivation occurs over a 60 min period. Thus, it is concluded that the Poly1-Cl beads severely impair those particles of poliovirus not inactivated outright such that they become inactivated in the

reservoir in the presence of very low concentrations of free chlorine. This two step action of the halogen on the infectivity of the virus particles permits higher levels of inactivation of poliovirus even in a gravity fed system. In other experiments more than 100 L of poliovirus suspension at 4 logs/mL were passed through 20 g of the Poly1-Cl beads in 10 L increments without any breakthrough of viable virus.[18]

Table 14 presents the data obtained for stability in storage of the Poly1-Cl beads under dry, vacuum conditions.

Table 14. Stability toward Loss of Chlorine for Poly1-Cl Beads in Dry Storage

Storage time (d)	% Cl loading	% Loss of Cl
0	17.16	---
10	17.15	0.06
16	17.13	0.17
23	16.93	1.34
90	16.23	5.42

Only about 5.4% of the chlorine was lost over a 90 d period. Since it has been shown that the biocidal beads function very well even at a chlorine loading of only 10.5%, it can be concluded that shelf-life storage time should not be a problem. However, it has been observed that the stability of the beads (as measured by Cl content) is less if moist conditions are present in a storage container.

Table 15 shows the results of the neutralization/recharge cycling experiment.

Table 15. Neutralization and Recharge Cycling for Poly1-Cl Beads

Cycle	pH[a]	% Cl loading
0	ND[b]	17.4
1	8.60	17.3
55	8.46	16.6
75	8.50	15.9
100	8.51	15.3

[a] The pH was adjusted during chlorination to about 8.5 so as to maintain the possibility of a constant chlorine charge.

[b] No determination, but the same chlorination conditions were employed for the original sample.

It was observed that there was a small loss of regenerability (about 12%) over the 100 cycles of neutralization/recharge. This indicates that the hydantoin ring is slowly decomposing over time which is expected under alkaline conditions.[19] However, the beads clearly would function well as biocides with 15.3% of the chlorine still remaining after 100 recharge cycles.

The mechanism of biocidal action of the Poly1-Cl and Poly1-Br beads has not yet been proven. However, it is clearly not related to dissociation of the N-X bond to form free halogen in the water. Less than 0.1 mg/L of free chlorine can be detected in the effluent of water passed continuously through

columns containing the Poly1-Cl. At a concentration that low, a much longer contact time (many min) would be required to achieve the inactivations observed for Poly1-Cl columns (a few s). We suggest that most probably a contact mechanism is involved in which upon collision of a pathogenic cell with a bead, Cl^+ (or Br^+) is directly transferred to the cell. Then the X^+ penetrates the cell wall and oxidizes targets within the cell or viral particle, causing inactivation of the organism. The number of such transfer events necessary to inactivate a single cell is open to question. The reason why the contact times necessary for cell inactivation are so short for the Poly1-Cl and Poly1-Br beads is that the effective concentration of the biocide is very large, in contrast to the concentrations (a few mg/L) which can be employed safely for water-soluble biocides which can require multiple min for inactivations.

Conclusions

The halogenated poly-styrenehydantoin beads addressed in this work, which were produced entirely by heterogeneous reactions, surprisingly contained halogen loadings comparable to those obtained earlier for an amorphous material produced primarily with homogeneous reaction steps. The porous beads provide inactivation times on the order of a few s for all pathogens tested in these laboratories thus far except poliovirus. In the case of poliovirus it appears that the plaque forming units are severely damaged upon passing through a column of Poly1-Cl beads, and they are subsequently inactivated in the presence of a very low concentration (<0.1 mg/L) of free chlorine in the effluent water. The beads are stable to halogen loss over months of dry storage, and they can be recharged numerous times by

exposure to free halogen. Thus it would appear that they should be very useful in water purification devices to be employed in producing potable water.

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RECOMMENDATIONS

The biocidal efficacies of the N-halamine functionalized polymeric materials Nylon, polyester, polyurethane paint, and polystyrene beads prepared in this project have been conclusively demonstrated. The efficacies against chemical agents need to be tested. It is recommended that the USAF especially consider the use of the biocidal polyurethane paint for medical facilities and food preparation areas to minimize infections of personnel and as a defense mechanism against biological (and possibly chemical) agents. It is also strongly recommended that the USAF consider the use of the biocidal polystyrene beads in cartridge filters for the treatment of field water in remote environments.